Infectious Tenosynovitis: Serologic and Histopathologic Response after Experimental Infection with a Connecticut Isolate

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SUMMARY

An infectious agent was isolated from leg tendons of broiler breeders with clinical tenosynovitis. The agent grew well on the chorioallantoic membrane (CAM) of embryonating chicken eggs and was filterable through a 0.22-μ filter. Typical gross and microscopic lesions of tenosynovitis could be reproduced in broiler chicks via oral, subcutaneous, or intra-abdominal routes, as well as in contact control chicks. Precipitating antibodies against the homologous agent as well as two other avian reoviruses were present beginning 14 days postinoculation. Negative control chicks developed no lesions or antibodies. The isolate showed antigenic identity with three known avian reoviruses when tested against antiserum in the agar-gel precipitin (AGP) test.

INTRODUCTION

Infectious tenosynovitis has been reported by Olson and Solomon (10), who reported the cause to be a viral arthritis agent. This agent has been characterized as a reovirus by Olson and Kerr (8). Incidence of tenosynovitis was reported on by Dalton and Henry (1) in England in 1967, by Rossi et al. (15) in Italy in 1969, by Krasselt and Voute (7) in the Netherlands in 1969, and by Johnson and van der Heide (5) in Maine in 1971. Kerr and Olson (6) reproduced gross and microscopic lesions of tenosynovitis with the viral arthritis agent after inoculation in the footpad. One contact control chicken also showed swelling of the tarsal extensor...
Table 1. AGP reactions of serum samples from chickens infected with tenosynovitis virus isolate S1133.

<table>
<thead>
<tr>
<th>Days post- inoculation</th>
<th>Antigen</th>
<th>Route of inoculation</th>
<th>Negative controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oral</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>7</td>
<td>S1133</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>UMI-203</td>
<td>1/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>WVU 2937</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>14</td>
<td>S1133</td>
<td>3/10</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>UMI-203</td>
<td>3/10</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>WVU 2937</td>
<td>2/10</td>
<td>10/10</td>
</tr>
<tr>
<td>21</td>
<td>S1133</td>
<td>3/10</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>UMI-203</td>
<td>4/10</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>WVU 2937</td>
<td>2/10</td>
<td>ND</td>
</tr>
<tr>
<td>28</td>
<td>S1133</td>
<td>9/10</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>UMI-203</td>
<td>10/10</td>
<td>5/5</td>
</tr>
<tr>
<td>53</td>
<td>S1133</td>
<td>8/10</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>UMI-203</td>
<td>9/10</td>
<td>ND</td>
</tr>
</tbody>
</table>

aNot done.

tendons. Johnson (4) reported isolation of a viral agent, designated UMI-203, from tendon material of chickens with clinical tenosynovitis. The UMI-203 isolate was reported by Petek (12) as belonging in the reovirus group on the basis of physicochemical characteristics. Johnson (4) reproduced the clinical disease by inoculating 2-day-old chicks with the UMI-203 isolate via the intramuscular, footpad, and intraperitoneal routes. Intranasally infected and contact control birds, however, showed no clinical or serologic response. Olson and Khan (9) reported in 1972 that the Fahey-Crawley virus, a reovirus, was capable of producing inflammatory changes in the digital flexor tendon sheaths upon intranasal inoculation in chickens. This strain was also described as a reovirus as a result of physicochemical characterizations by Petek and co-workers (11,13,14).

In 1971, tenosynovitis was diagnosed in 7-week-old broiler

Table 2. Precipitating antibodies of three types found at three ages in two groups of chickens held in contact with tenosynovitis-infected chickens.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>29</th>
<th>36</th>
<th>54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>S1133</td>
<td>UMI-203</td>
<td>S1133</td>
</tr>
<tr>
<td>Contact controls with orally infected chickens</td>
<td>2/5a</td>
<td>1/5</td>
<td>3/7</td>
</tr>
<tr>
<td>Contact controls with intra-abdominally infected chickens</td>
<td>3/5</td>
<td>3/5</td>
<td>7/10</td>
</tr>
</tbody>
</table>

aNo. of birds with antibody type/no. of birds examined.
bNot done.
breeders in Connecticut. Gross lesions consisted of swelling of tendon sheaths on the shanks and above the hock joints. Sera from these chickens were negative for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies by the agglutination and hemagglutination-inhibition tests.

A clear yellow serous fluid existed between the tendons, while in some birds the tendons above the hock joints were hemorrhagic, frayed, and in the process of rupturing. Chickens were reluctant to walk, and had a painful gait when forced.

A viral agent was isolated from the leg lesions. Clinical, pathologic, and serologic responses are described upon reinoculation of this viral isolate into broiler chickens via oral, subcutaneous, and intra-abdominal routes, as well as by contact exposure.

**MATERIALS AND METHODS**

**Virus isolation.** Leg-tendon material from 7-week-old broiler breeder chickens with acute tenosynovitis was triturated in a Ten Broeck grinder. Penicillin (approximately 10,000 units) and streptomycin (approximately 10 mg) were added per ml of homogenate, and 9-to-10-day embryonating SPF chicken eggs were inoculated on the chorioallantoic membrane (CAM). CAM material was harvested 3 days postinoculation. Harvested CAM material was triturated in a Ten Broeck grinder with broth, penicillin, streptomycin (and sometimes mycostatin) added, and serial passages were made on CAM's of 9-to-10-day embryonating SPF chicken eggs. CAM's showed a consistent lesion pattern, a gray edematous swelling of the entire area of the dropped CAM, with death of the embryos of most infected eggs.

**Antigen for agar-gel precipitin test.** Homogenized CAM material from one of the various passages of the above-described isolate, designated S1133, was used in the agar-gel precipitin (AGP) test, as well as CAM homogenate from embryonating eggs infected with Olson's viral arthritis agent, WVU 2937, and the UMI-203 isolate from Maine.

The AGP test was performed with 1% Special Agar Noble solution with 8% salt at pH 7.4. To prepare such a solution, 5.0 g of Special Agar Noble (Difco) and 40 mg NaCl are added to 467 ml of standard phosphate buffer solution at pH 7.4. After the ingredients are dissolved by boiling, 7.0 ml of the solution is poured into each of 100 × 15-mm plastic petri dishes and allowed to solidify. These plates can be stored for at least 1 month in cannisters with moist paper towels added, and refrigerated.
Fig. 1. Cross section of metatarsal tendon sheath of IA-inoculated chicken, 5 weeks postinoculation, showing proliferative nonpurulent tenosynovitis with foci of infiltrating mononuclear cells. H&E, ×32.

Fig. 2. Cross section of metatarsal tendon sheath of SC-inoculated chicken, 5 weeks postinoculation. Notice the marked fibrosis and thickened tendon sheath. H&E, ×32.
Table 3. Average weight per chicken at 5 weeks of age after experimental infection with tenosynovitis at 1 day old.

<table>
<thead>
<tr>
<th>Pen no.</th>
<th>Route of inoculation</th>
<th>Isolate used</th>
<th>Av. weight at 5 weeks (g) / (number of chickens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Negative controls</td>
<td>---</td>
<td>679 (5)</td>
</tr>
<tr>
<td>2</td>
<td>Subcutaneous</td>
<td>S1133</td>
<td>567 (10)</td>
</tr>
<tr>
<td>3</td>
<td>Intra-abdominal</td>
<td>S1133</td>
<td>461 (11)</td>
</tr>
<tr>
<td>5</td>
<td>Contact controls</td>
<td>---</td>
<td>605 (10)</td>
</tr>
<tr>
<td>5</td>
<td>Intra-abdominal</td>
<td>UMI-203</td>
<td>415 (3)</td>
</tr>
<tr>
<td>6</td>
<td>Subcutaneous</td>
<td>UMI-203</td>
<td>484 (10)</td>
</tr>
</tbody>
</table>

Groups of 6 wells 3.0 mm in diameter were cut around a center well with a 3.0-mm distance between the center well and peripheral wells. The center wells were filled with antigen, and the peripheral wells with serum samples, and the precipitation reactions were read after approximately 24 hours of incubation at room temperature in a humidified atmosphere.

**Experimental design.** Three groups of day-old broiler breeder chickens were inoculated via oral, subcutaneous (SC), or intra-abdominal (IA) routes with approximately $10^{2.2}$ ELD$_{50}$ of tenosynovitis virus isolate S1133. A 4th group was held as negative controls in a separate building. Contact controls were placed in the pens of the orally and IA-infected groups.

At 1 day of age, 20 chickens were tested for precipitating maternal antibodies. Blood serum samples of the various groups were tested periodically for the presence of precipitating antibodies. Results are shown in Tables 1 and 2.

Chickens were weighed when killed, and cross sections were made of the metatarsal digital flexor tendons from a number of chickens that showed clinical tenosynovitis. Sections were also made from negative and contact control chickens, although gross lesions were absent in these latter two groups. Tissues were sectioned after fixing in 10% formalin-saline, and were stained with hematoxylin and eosin. Table 3 shows average weights of chickens in infected, contact control, and negative control groups.

**RESULTS**

**Serology.** At 1 day old, 1 of 20 chicks tested showed a positive AGP reaction against the UMI-203 isolate but was negative against the S1133 isolate and viral arthritis agent WVU 2937.

Precipitating antibodies were found beginning at 2 weeks of age in the chickens infected orally, SC, and IA. At 1 week of age, 1 orally infected but also 1 negative control chicken showed a posi-
Fig. 3. Cross section of digital flexor tendon sheath of contact control chicken, 5 weeks old. Proliferative tenosynovitis with infiltration of mononuclear cells. H&E, ×32.

Fig. 4. Cross section of digital flexor tendon sheath of negative control chicken, 5 weeks old. H&E, ×32.
tive AGP reaction. These may be regarded as a remnant of ma-
ternal antibody. Precipitating antibodies were also found in the
serum of contact control chickens at 4 and 5 weeks of age (Table
2). Pooled serum samples from infected and negative control
chickens were negative for Mycoplasma gallisepticum and Myco-
plasma synoviae by the agglutination test.

**Weight.** Chickens infected by either oral, SC, or IA routes
showed a marked growth inhibition compared with growth of nega-
tive control chickens. Contact control chickens also showed a
growth depression compared with growth of negative control
chickens, though to a lesser degree than in inoculated chickens.

**Histopathology.** Microscopic examination showed that birds
infected SC and IA had a proliferative nonpurulent tenosynovitis,
with foci of infiltrating mononuclear cells, at 5 weeks postinocula-
tion (Fig. 1).

At 5 weeks postinoculation, chickens infected by SC showed
marked fibrosis in the thickened tendon sheaths. These lesions ap-
peared more chronic and less violent than in the IA-infected birds
(Fig. 2).

At 7 1/2 weeks postinoculation, orally infected chickens showed
chronic fibrosis of the tendon sheaths, with fibrous tissue invading
the tendons and resulting in ankylosis and immobility.

One contact control chicken in the orally infected group and 2
contact controls in the IA-infected group showed a marked pro-
liferative tenosynovitis with infiltration of mononuclear cells (Fig.
3).

None of the negative control birds showed any gross or micro-
scopic lesions in the examined digital tendon cross sections (Fig.
4).

**DISCUSSION**

Serologic reactions and microscopic lesions indicate that oral
and contact transmission of infectious tenosynovitis are possible,
resulting in the formation of precipitating antibodies and the de-
velopment of lesions in the tendon sheaths of infected chickens.

When tested against an anti-S1133 serum in the AGP test, the
Connecticut tenosynovitis isolate, S1133, showed a line of identity
with Olson's viral arthritis agent WVU 2937, Johnson's UMI-203,
and also reo 25, isolated by Deshmukh and Pomeroy (2,3).

Further identification studies of the S1133 isolate are in
progress.
REFERENCES